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(FILE 'HOME' ENTERED AT 16:15:25 ON 20 SEP 2006)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE,  
AQUALINE,  
AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,  
CAPLUS,  
CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS,  
DRUGB,  
DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 16:15:44 ON 20 SEP  
2006

SEA DEAMIDASE

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6 FILE AGRICOLA  
3 FILE ANABSTR  
1 FILE AQUASCI  
5 FILE BIOENG  
144 FILE BIOSIS  
5 FILE BIOTECHABS  
5 FILE BIOTECHDS  
24 FILE BIOTECHNO  
10 FILE CABA  
250 FILE CAPLUS  
3 FILE CONFSCI  
21 FILE DDFB  
8 FILE DDFU  
3 FILE DGENE  
7 FILE DISSABS  
21 FILE DRUGB  
11 FILE DRUGU  
70 FILE EMBASE  
24 FILE EMBIOWASE  
7 FILE FROSTI  
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25 FILE LIFESCI  
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1 FILE NTIS  
2 FILE OCEAN  
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1 FILE RDISCLOSURE  
79 FILE SCISEARCH  
62 FILE TOXCENTER  
91 FILE USPATFULL  
9 FILE USPAT2  
15 FILE WPIDS  
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L1

QUE DEAMIDASE

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FILE 'CAPLUS, MEDLINE, BIOSIS, SCISEARCH, EMBASE, TOXCENTER, PASCAL,  
LIFESCI, BIOTECHNO' ENTERED AT 16:19:02 ON 20 SEP 2006

L2 892 S L1  
L3 31 S L2 AND TRANSGlutAMINASE  
L4 16 S L2 AND (CHRYSEOBACTERIUM OR FLAVOBACTERIUM OR  
EMPEDOBACTER O  
L5 10 DUP REM L4 (6 DUPLICATES REMOVED)

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=> d 15 ibib ab 1-10

L5 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2004:523093 CAPLUS  
DOCUMENT NUMBER: 141:84625  
TITLE: Cloning, characterization and sequence of  
protein-deamidating enzyme from  
Chryseobacterium and uses in the food industry  
INVENTOR(S): Yamaguchi, Shotaro  
PATENT ASSIGNEE(S): Amano Enzyme Inc., Japan  
SOURCE: U.S., 28 pp., Cont.-in-part of U.S. 6,251,651.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 3  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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US 6756221	B1	20040629	US 2000-727769	20001204
US 6251651	B1	20010626	US 1999-324910	19990603
US 2004072318	A1	20040415	US 2001-793495	20010227
US 6770469	B2	20040803		
US 2004166558	A1	20040826	US 2004-815751	20040402
US 2004175799	A1	20040909	US 2004-815774	20040402
JP 2005052158	A2	20050303	JP 2004-342158	20041126
JP 3769289	B2	20060419		
PRIORITY APPLN. INFO.:			US 1999-324910	A2 19990603
			JP 1999-345044	A 19991203
			JP 1998-173940	A 19980604
			JP 2000-368983	A3 20001204
			US 2000-727769	A3 20001204
			US 2001-793495	A3 20010227

AB The invention involves an enzyme which removes amido groups from proteins and releases side-chain carboxyl groups and ammonia. The enzyme acts directly on amido groups without cleaving peptide bonds and without crosslinking a protein substrate and is therefore called protein deamidating enzyme. In addition, this invention claims methods for the production of an enzyme, which comprise culturing in a medium a strain that belongs to a bacterium classified into Cytophagales or Actinomycetes, or a new bacterium Chryseobacterium strain 9670, and has the ability to produce an enzyme having property to deamidate amido groups in protein, thereby effecting production of the enzyme, and subsequently collecting the enzyme from the culture mixture The invention also claims polypeptide and

nucleotide sequences for the enzyme from Chryseobacterium strain 9670. A method for the modification of an enzyme using the native or recombinant protein deamidating enzyme and a method and composition for the modification of a protein substrate using the native or recombinant protein deamidating enzyme are also claimed. Methods for use of a recombinant protein deamidating enzyme involve using a gene which encodes the enzyme, a recombinant vector which contains the gene, a transformant transformed with the vector and a method in which the transformant is cultured in a medium to effect production of the protein-deamidating enzyme and then the protein-deamidating enzyme is collected. Uses of the protein deamidating enzyme include for improvement of proteins in food and alterations in protein and food properties such as solubility, foamability, and emulsifying ability. A purified protein deamidating enzyme was obtained from a new microorganism, Chryseobacterium strain 9670, and its activity measured by release of ammonia from Cbz-Gln-Gly and casein assay substrates. Protease and transglutaminase activities of the purified protein deamidating enzyme were not detected. Several protein substrates, including wheat gluten, egg white protein, and soybean protein, were treated with the Chryseobacterium deamidating enzyme and pH-solubility curves of the substrates were determined

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L5 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
 ACCESSION NUMBER: 2003:702816 CAPLUS  
 DOCUMENT NUMBER: 139:196614  
 TITLE: Functional improvement milk protein by enzymic deamidation  
 INVENTOR(S): Matsumura, Yasuo; Mori, Tomohiko  
 PATENT ASSIGNEE(S): Amano Enzyme Inc., Japan  
 SOURCE: Jpn. Kokai Tokkyo Koho, 11 pp.  
 CODEN: JKXXAF  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Japanese  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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JP 2003250460	A2	20030909	JP 2002-57954	20020304
PRIORITY APPLN. INFO.:			JP 2002-57954	20020304

AB The milk protein (mol.-weight,  $\geq 5,000$ ) is incubated with deamidase of microorganism selected from Chryseobacterium, Flavobacterium, Empedobacterium, etc., for deamidation but not

simultaneous crosslinking. The deamidated milk protein has lower allergenicity, high surface activity, better flavor which is associated with binding of low-mol.-weight off-flavors, higher hydrophobicity, better binding of low-mol.-weight substance such as retinol for enhanced bioavailability, higher susceptibility to protease such as pepsin, etc.

L5 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1  
 ACCESSION NUMBER: 2001:432945 CAPLUS  
 DOCUMENT NUMBER: 135:42762  
 TITLE: Protein-deamidating enzyme, microorganism producing the same, gene encoding the same, production process  
 therefor, and use thereof  
 INVENTOR(S): Yamaguchi, Shotaro  
 PATENT ASSIGNEE(S): Amano Enzyme Inc., Japan  
 SOURCE: Eur. Pat. Appl., 43 pp.  
 CODEN: EPXXDW  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 3  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1106696	A1	20010613	EP 2000-310768	20001204
EP 1106696	B1	20050706		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001218590	A2	20010814	JP 2000-368983	20001204
JP 3696500	B2	20050921		
US 2004166558	A1	20040826	US 2004-815751	20040402
JP 2005052158	A2	20050303	JP 2004-342158	20041126
JP 3769289	B2	20060419		
PRIORITY APPLN. INFO.:			JP 1999-345044	A 19991203
			JP 1998-173940	A 19980604
			US 1999-324910	A3 19990603
			JP 2000-368983	A3 20001204
			US 2001-793495	A3 20010227

AB The invention involves an enzyme which removes amido groups from proteins and releases side-chain carboxyl groups and ammonia. The enzyme acts directly on amido groups without cleaving peptide bonds and without crosslinking a protein substrate and is therefore called protein deamidating enzyme. The invention also claims polypeptide and nucleotide sequences for the enzyme. In addition, this invention claims methods for the production of an enzyme, which comprise culturing in a medium a strain that

belongs to a bacterium classified into Cytophagales or Actinomycetes,  
or a  
new bacterium Chryseobacterium sp. Number 9670 belonging to the  
genus Chryseobacterium, and has the ability to produce an enzyme  
having property to deamidate amido groups in protein, thereby effecting  
production of the enzyme, and subsequently collecting the enzyme from  
the  
culture mixture A method for the modification of an enzyme using the  
native  
or recombinant protein deamidating enzyme and a method and composition  
for the  
modification of a protein substrate using the native or recombinant  
protein deamidating enzyme are also claimed. Methods for use of a  
recombinant protein deamidating enzyme involve using a gene which  
encodes  
the enzyme, a recombinant vector which contains the gene, a  
transformant  
transformed with the vector and a method in which the transformant is  
cultured in a medium to effect production of the protein-deamidating  
enzyme  
and then the protein-deamidating enzyme is collected. Uses of the  
protein  
deamidating enzyme include for improvement of proteins in food and  
alterations in protein and food properties such as solubility,  
foamability, and  
emulsifying ability. A purified protein deamidating enzyme was  
obtained  
from a new microorganism, Chryseobacterium strain 9670, and its  
activity measured by release of ammonia from Cbz-Gln-Gly and casein  
assay  
substrates. Protease and transglutaminase activities of the purified  
protein deamidating enzyme were not detected. Several protein  
substrates,  
including wheat gluten, egg white protein, and soybean protein, were  
treated with the Chryseobacterium deamidating enzyme and  
pH-solubility curves of the substrates were determined

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR  
THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L5 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2000:84446 CAPLUS

DOCUMENT NUMBER: 132:119242

TITLE: Protein-deamidating enzyme and its  
Chryseobacterium gleum gene and uses in the  
food industry

INVENTOR(S): Yamaguchi, Shotaro; Matsuura, Akira

PATENT ASSIGNEE(S): Amano Pharmaceutical Co., Ltd., Japan; Amano Enzyme  
Inc.

SOURCE: Eur. Pat. Appl., 57 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 976829	A2	20000202	EP 1999-304367	19990604
EP 976829	A3	20000216		
EP 976829	B1	20040602		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000050887	A2	20000222	JP 1999-158703	19990604
JP 3609648	B2	20050112		
US 2004072318	A1	20040415	US 2001-793495	20010227
US 6770469	B2	20040803		
US 2004166558	A1	20040826	US 2004-815751	20040402
PRIORITY APPLN. INFO.:			JP 1998-173940	A 19980604
			US 1999-324910	A3 19990603
			JP 1999-345044	A 19991203
			US 2001-793495	A3 20010227

AB An enzyme is provided which has an activity to release side chain carboxyl groups and ammonia from a protein by acting upon side chain amido groups in the protein. This invention relates to a method for the production of an enzyme, which comprises culturing in a medium a strain that belongs to a bacterium classified into Cytophagales or Actinomycetes has the ability to produce an enzyme having a property to deamidate amido groups in protein, thereby effecting production of said enzyme, and subsequently collecting said enzyme from the culture mixture. The gene encoding the enzyme was isolated and sequenced from *Chryseobacterium gleum*, and shown to encode a 319-residue protein including a 134-amino acid prepro moiety. The protein-deamidating enzyme can be used as a reagent for use in the functional modification of protein, i.e., in protein engineering. Deamidation of plant and animal food proteins results in improved functional properties (solubility and dispersibility) for the preparation of numerous food products. The protein-deamidating enzyme can also be used as a reaction controlling agent for transglutaminase.

L5 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2  
 ACCESSION NUMBER: 2000:552802 CAPLUS  
 DOCUMENT NUMBER: 133:277885  
 TITLE: A novel protein-deamidating enzyme from *Chryseobacterium proteolyticum* sp. nov., a newly isolated bacterium from soil  
 AUTHOR(S): Yamaguchi, Shotaro; Yokoe, Masaaki  
 CORPORATE SOURCE: Gifu R & D Center, Amano Pharmaceutical Co., Ltd.,

SOURCE: Gifu, 509-0108, Japan  
Applied and Environmental Microbiology (2000),  
66(8),

3337-3343

CODEN: AEMIDF; ISSN: 0099-2240

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A novel protein-deamidating enzyme, which has potential for industrial applications, was purified from the culture supernatant of *Chryseobacterium proteolyticum* strain 9670T isolated from rice field soil in Tsukuba, Japan. The deamidating activities on carboxybenzoxy (Cbz)-Gln-Gly and caseins and protease activity were produced synchronously by the isolate. Both deamidating activities

were

eluted as identical peaks separated from several proteases by phenyl-Sepharose

chromatog. of the culture supernatant. The enzyme catalyzed the deamidation of native caseins with no protease and transglutaminase activities. Phenotypic characterization and DNA analyses of the

isolate

were performed to determine its taxonomy. Physiol. and biochem. characteristics, 16S rRNA gene sequence anal., and DNA-DNA relatedness data indicated that the isolate should be placed as a new species belonging to the genus *Chryseobacterium*. The isolate showed no growth on MacConkey agar and produced acid from sucrose. The levels of DNA-DNA relatedness between the isolate and other related strains were less than 17%. The name *Chryseobacterium proteolyticum* is proposed for the new species; strain 9670 is the type strain (=FERM P-17664).

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS

FORMAT RECORD. ALL CITATIONS AVAILABLE IN THE RE

L5 ANSWER 6 OF 10 MEDLINE on STN

ACCESSION NUMBER: 84195167 MEDLINE

DOCUMENT NUMBER: PubMed ID: 6371900

TITLE: Proline specific endopeptidase.

AUTHOR: Yoshimoto T; Tsuru D

SOURCE: Tanpakushitsu kakusan koso. Protein, nucleic acid, enzyme,

(1984 Feb) Vol. 29, No. 2, pp. 127-33. Ref: 38

Journal code: 0413762. ISSN: 0039-9450.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: Japanese

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198406

ENTRY DATE: Entered STN: 19 Mar 1990

Last Updated on STN: 3 Mar 2000

Entered Medline: 19 Jun 1984

L5 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 1980:464192 CAPLUS



DOCUMENT NUMBER: 93:64192  
TITLE: Nicotinamide deamidase from  
Flavobacterium peregrinum  
AUTHOR(S): Tanigawa, Yoshinori; Shimoyama, Makoto; Ueda, Iwao  
CORPORATE SOURCE: Dep. Biochem., Shimane Med. Univ., Shimane, 693,  
Japan  
SOURCE: Methods in Enzymology (1980), 66(Vitam. Coenzymes,  
Pt.  
E), 132-6  
CODEN: MENZAU; ISSN: 0076-6879  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: English  
AB A review with 9 refs.

L5 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4  
ACCESSION NUMBER: 1973:107633 CAPLUS  
DOCUMENT NUMBER: 78:107633  
TITLE: Purification and properties of nicotinamide  
deamidase from Flavobacterium  
peregrinum  
AUTHOR(S): Tanigawa, Yoshinori; Shimoyama, Makoto; Dohi,  
Kakuo;  
Ueda, Iwao  
CORPORATE SOURCE: Dep. Med. Chem., Osaka Med. Coll., Osaka, Japan  
SOURCE: Journal of Biological Chemistry (1972), 247(24),  
8036-42  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Nicotinamide deamidase from *F. peregrinum* was purified .apprx.  
210-fold with the use of protamine and  $\text{MgCl}_2$  treatments, acetone  
fractionation,  $(\text{NH}_4)_2\text{SO}_4$  fractionation, Sephadex G-200, and CM-Sephadex  
column chromatography. The optimum pH of the purified nicotinamide  
deamidase is 6.5, and the  $K_m$  value for nicotinamide is  $0.2\mu\text{M}$ .  
The molecular weight was estimated by means of gel filtration to be  
48,000. The enzyme activity of the crude preparation was increased by  
2.5-fold by addition of  $\text{Mn}^{2+}$  at a concentration of 10mM followed by  
incubation at  $37^\circ$  for 30 min. The purified enzyme preparation was  
inactivated by dialysis in the absence of  $\text{Mn}^{2+}$ , and no recovery of  
enzyme  
activity was possible even when  $\text{Mn}^{2+}$  or  $\text{Mn}^{2+}$  plus cysteine was added to  
the reaction mixture. When the enzyme was dialyzed overnight in the  
presence of  $\text{Mn}^{2+}$ , almost all of the enzyme activity was retained. A  
dialysis of the enzyme against 0.01M maleate buffer (pH 6.5) containing  
 $5\mu\text{M}$   $\text{HgCl}_2$  resulted in complete retention of enzyme activity when 50mM  
cysteine was added to the reaction mixture.  $\text{CuSO}_4$  and  $\text{SnCl}_2$  also  
showed  
effects similar to that of  $\text{HgCl}_2$ , but their potency for enzyme  
stability  
was small.  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Fe}^{2+}$  did not show any effect on  
enzyme  
activity. All the nicotinamide analogs having a trivalent N in the  
pyridine ring showed a competitive inhibition of nicotinamide  
deamidation,  
whereas compounds having a tetravalent N seemed to have no effect.  
From

these results the structure of the enzyme-metal-substrate complex proposed was that of a triangle with each of the 3 components being bound to each other.

L5 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 5  
ACCESSION NUMBER: 1973:68603 CAPLUS  
DOCUMENT NUMBER: 78:68603  
TITLE: Nicotinamide deamidase of microorganisms isolated from rat stomach  
AUTHOR(S): Tanigawa, Yoshinori  
CORPORATE SOURCE: Dep. Med. Chem., Osaka Med. Coll., Takatsuki, Japan  
SOURCE: Bulletin of the Osaka Medical School (1972), 18(1), 30-47  
CODEN: BUOSA5; ISSN: 0030-6142  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Almost all stomach nicotinamide deamidase (I) activity was localized in the pars preventricularis of normal rats, but no activity was found there in the germ-free rats. I activities were found, in decreasing order of potency, in Flavobacterium peregrinum, Escherichia coli, Streptococcus faecalis, and Lactobacillus acidophilus. I from F. peregrinum was purified in the presence of Mn<sup>++</sup>, about 70-fold. The Km value for nicotinamide was  $2 \times 10^{-7}$ M and the pH optimum was 7.0. If purification was carried out in the absence of Mn<sup>++</sup>, the enzyme activity decreased, with 1% recovery at the final step of purification. The purified I was inactivated through dialysis against a large volume of 0.01M malate buffer, pH 7.0, in the absence of Mn<sup>++</sup> and its activity was not recovered by the subsequent addition of Mn<sup>++</sup> or Mn<sup>++</sup> plus cysteine. If the dialysis was carried out in the presence of Mn<sup>++</sup> or Hg<sup>++</sup>, the activity remained intact. With Hg<sup>++</sup> this effect was only observed with cysteine in the reaction mixture. The half-maximum level of the Mn<sup>++</sup> and Hg<sup>++</sup> effects for enzyme stabilization during dialysis were  $4.5 \times 10^{-5}$ M and  $1.25 \times 10^{-6}$ M, resp. EDTA led to a decrease in enzyme activity but this decrease could be completely restored by Mn<sup>++</sup>.

L5 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1971:537838 CAPLUS  
DOCUMENT NUMBER: 75:137838  
TITLE: Nicotinamide deamidation by microorganisms in rat stomach  
AUTHOR(S): Shimoyama, Makoto; Tanigawa, Yoshinori; Ito, Toichi;  
Murashima, Ryuzo; Ueda, Iwao; Tomoda, Tsunesuke  
CORPORATE SOURCE: Dep. Med. Chem., Osaka Med. Coll., Takatsuki, Japan  
SOURCE: Journal of Bacteriology (1971), 108(1), 191-5  
CODEN: JOBAAY; ISSN: 0021-9193  
DOCUMENT TYPE: Journal

LANGUAGE: English

AB The bacterial species in the pars preventricularis were identified as *Flavobacterium peregrinum*, *Escherichia coli*, *Streptococcus faecalis*, and *Lactobacillus acidophilus*, listed in order of decreasing deamidase activity. Nicotinamide-7-<sup>14</sup>C ingested into rat stomach was rapidly deamidated to nicotinic acid. These results contribute to the accumulated evidence that microorganisms present in the pars preventricularis of rat stomach are responsible for the deamidation of nicotinamide to nicotinic acid, a known precursor of mammalian pyridine nucleotides.

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